Published in final edited form as:
Wiley Interdiscip Rev Syst Biol Med. 2012 ; 4(1): 15–37. doi:10.1002/wsbm.157.

A Systems Biology Approach to Synovial Joint Lubrication in Health, Injury, and Disease

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Abstract

The synovial joint contains synovial fluid (SF) within a cavity bounded by articular cartilage and synovium. SF is a viscous fluid that has lubrication, metabolic, and regulatory functions within synovial joints. SF contains lubricant molecules, including proteoglycan-4 and hyaluronan. SF is an ultrafiltrate of plasma with secreted contributions from cell populations lining and within the synovial joint space, including chondrocytes and synoviocytes. Maintenance of normal SF lubricant composition and function are important for joint homeostasis. In osteoarthritis, rheumatoid arthritis, and joint injury, changes in lubricant composition and function accompany alterations in the cytokine and growth factor environment and increased fluid and molecular transport through joint tissues. Thus, understanding the synovial joint lubrication system requires a multi-faceted study of the various parts of the synovial joint and their interactions. Systems biology approaches at multiple scales are being used to describe the molecular, cellular, and tissue components and their interactions that comprise the functioning synovial joint. Analyses of the transcriptome and proteome of SF, cartilage, and synovium suggest that particular molecules and pathways play important roles in joint homeostasis and disease. Such information may be integrated with physicochemical tissue descriptions to construct integrative models of the synovial joint that ultimately may explain maintenance of health, recovery from injury, or development and progression of arthritis.

Keywords

Musculo-skeletal system; High throughput screening; Proteomics; Mathematical models; Homeostasis

1. Synovial Joint Lubrication in Health, Injury, and Disease

1.A. General Overview

Synovial joints are the most common joints of the human body and allow load-bearing, low-friction, wear-resistant movement between apposing bone surfaces.[1] Synovial joints normally include a cavity filled with synovial fluid (SF). The SF cavity is surrounded by, articular cartilage covering the bone surfaces, and a fibrous capsule including the synovium, the inner lining.

SF has biomechanical, metabolic, and regulatory functions. SF is normally a clear, straw-colored, viscous liquid. In normal human knee joints, the volume of SF is ~1 ml.[2] One main function of SF is to lubricate cartilage in synovial joints, facilitating low-friction and low-wear articulation.[1] The molecular and cellular constituents within SF give rise to its unique properties and functions in maintaining joint homeostasis. SF is composed of a blood plasma dialysate and molecules secreted by cells lining and within the synovial joint space, including the lubricant molecules, hyaluronan (HA) and proteoglycan 4 (PRG4, also known as lubricin and superficial zone protein).[3-5]Additionally, SF serves metabolic functions, facilitating the transport of nutrients, waste products, and other metabolites to and from synovial tissues, as well as enzymes that act upon and within these tissues. Finally, SF contains soluble molecules, such as morphogens, growth factors, and cytokines, which mediate communication between cell populations in the joint.[6-9]

Joint disease and injury can result in pain and dysfunction of synovial joints. Osteoarthritis (OA) is a degenerative disease that results in destructive changes tojoint structures including cartilage, synovium, and bone. OA has been classified as idiopathic or secondary to other factors, such as joint trauma and congenital diseases. Knee OA has been classified according to clinical, laboratory, and radiographic criteria.[10] Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by joint swelling, joint tenderness, and destruction of synovial joints.[11] Synovitisis a key clinical feature of RA, and together with other criteria including the number of joints involved, duration of symptoms, and test results for serology and acute-phase reactants, can be used to classify RA.[11, 12] Traumatic joint injury can result in cartilage damage, articular and bone fractures, damage to soft tissues such as ligaments and menisci, and lesions in the joint capsule and synovium. Such damage is associated with a number of mechanical and biological changes in the synovial joint that contribute to the development of post-traumatic OA.[13-15]

The composition and function of SF are altered in joint injury and disease, both due to changes directly of the SF and changes in tissues of the synovial joint. SF is in direct physical contact with cartilage and synovium, and in some joints, meniscus and ligament. SF interacts with and mediates interactions between synovial joint tissues. These tissues may themselves be altered in injury and disease. Changes in cellular metabolism and structure in these tissues may be reflected by changes in SF composition and function. Such changes in SF may result in a reduced ability to lubricate articulating cartilage and a catabolic environment within the joint, together contributing to joint deterioration. Alterations in joint tissues may be detrimental, propelling the SF to an aberrant state and leading to joint pathology. Thus, the disease-associated changes observed in SF likely are both exacerbated by and contribute to pathology of the synovial joint.

The objectives of this review are to provide an overview of (1) synovial joint lubrication in health, injury, and disease, with a focus on normal SF composition, formation, and rheological and tribological properties, and their alterations with joint injury and disease, and (2) high-throughput transcriptomic and proteomic studies of synovial joint cells and

tissues that enable integrative models of synovial joint lubrication and a systems biology approach to studying synovial joint lubrication in health, injury, and disease.

1.B. SF Composition

1.B.i. Plasma Proteins—A major component of SF composition is proteins derived from plasma. Blood plasma and SF share many similarities in their protein composition,[16, 17] with a superimposed effect of the sub-synovium and synovium selectively hindering large plasma proteins from entering the synovial joint space from the vasculature. Total protein concentration in normal human SF is 19–28 mg/ml, nearly one-third of that in plasma.[2, 18, 19] Quantitatively, the major protein species in SF is albumin, at a concentration of ~12 mg/ml.[19] Other major protein components of normal SF include β_1, γ, α_1,and α_2 globulins, each at concentrations of 1–3 mg/ml in normal SF.[19] The size of plasma proteins is a major determinant of their filtration properties through the synovial membrane and entry into SF, and thus their concentrations in SF relative to those in plasma. Large molecular weight plasma proteins, such as fibrinogen (340kDa), are at relatively low concentrations in SF; in contrast, small molecular weight plasma proteins such as albumin (69 kDa) and transferrin (90 kDa), are in relatively high concentrations in SF (the concentration of albumin being ~37% of that in plasma).[19-21]

The protein content and concentration in SF is increased with joint inflammation. Total protein concentration in SF from patients with OA, rheumatoid arthritis RA, and traumatic arthritis is higher than normal,[19, 21-23] indicating structural and functional changes in the synovium with joint injury and disease. Synovial inflammation, as occurs in many joint diseases, compromises the ability of synovium to selectively retain and filter proteins. For example, SF from RA patients has high levels of globulins and glycoproteins, large molecular weight proteins normally not found in normal SF. The distribution of proteins in SF of RA patients is also altered and more closely resembles that in serum. Large plasma proteins, such as β_2 macroglobulin (1,000kDa), fibrinogen (340kDa), β_1 lipoprotein (3,200kDa), α_2 macroglobulin (820kDa), and α_2 glycoprotein (1,000kDa), are present in SF at increased concentrations and amounts in RA patients.[20, 21]

1.B.ii. Lubricant Molecules—Lubrication of articulating cartilage surfaces by SF is mediated by several lubricant macromolecules synthesized and secreted by synovial cell populations and found in SF. HA[24] and PRG4[25, 26] are the primary lubricant macromolecules in SF and are present in normal SF at mean concentrations of ~3.2–4.1mg/ml[27-29]and ~0.035–0.24 mg/ml,[30-32]respectively. HA is a non-sulfated glycosaminoglycan composed of repeating disaccharide units of D-glucuronic acid and D-N-acetylglucosamine. HA in normal SF is present as a polydisperse population with a weight average molecular weight of 6–7 MDa, with the majority greater than 4MDa.[33] HA contributes to the viscosity of SF and provides outflow buffering.[34] Products of the *PRG4* gene, which include superficial zone protein (SZP) and lubricin, are mucinous glycoproteins with multiple O-linked β(1-3)Gal-GalNAc oligosaccharides that mediate boundary lubrication of articular cartilage.[35] SZP is a ~345 kDa glycoprotein synthesized and secreted by chondrocytes in the superficial zone of cartilage, and not from other depths of cartilage.[3] Lubricin is a ~220 kDa glycoprotein expressed by synovial fibroblasts and also present in SF.[4, 26, 36]

OA, RA, and injury are associated with characteristic changes in SF lubricant macromolecules (Table 1A). The mean concentrations of HA in pathological SF are lower than that in normal SF, with SF from OA patients ranging from ~1.2–2.2 mg/ml[27, 37, 38] and RA patients ranging from ~0.7–2.7 mg/ml.[27, 29, 37, 39, 40] The molecular weight distribution of HA in pathological SF is also altered, with a shift to lower molecular weight forms.[39] In an ovine meniscectomy model of osteoarthritis, cellular PRG4 immunostaining and mRNA

levels were decreased in degenerative cartilage compared to normal cartilage.[41] SF analysis following transection of the rabbit anterior cruciate ligament (ACL) and posterior cruciate ligament (PCL) revealed an association between decreased lubricin concentrations and boundary lubricating ability, increased elastase activity, and increased cartilage degradation.[42]

The effects of injury on the concentration of lubricin in SF appear variable. Lubricin concentrations in the SF of patients with ACL injury were reported to be lower compared to SF from the uninjured contralateral knee during the first several months post-injury, and increasing to contralateral values by~12 months.[31] However, SF lubricin concentrations in longitudinal samples from patients with recent severe knee injury decreased from the time of presentation (~15 days post-injury) to the time of arthroscopic surgery (~48 days post-injury).[43] SF lubricin concentrations may be altered post-injury due to changes in the rates of synthesis, secretion, degradation, and/or loss from the joint, as well as changes in SF volumes. The effects of injury on lubricin synthesis and secretion have been studied with experimental models of cartilage mechanical injury. In a bovine model, lubricin mRNA expression and protein synthesis and secretion increased in explants from superficial regions of cartilage with an intact articular surface after 2 days following injurious compression.[44] Further studies are needed to clarify the effects of injury on human SF lubricin concentrations. The absence of PRG4, both in patients with the genetic disease camptodactyly-arthropathy-coxa vara-pericarditis syndrome (CACP),[45] an autosomal recessive disease with loss of function mutations in the PRG4 gene, and in *PRG4*$^{/-}$ mice,[46] results in cartilage degeneration and synovial hyperplasia.

1.B.iii. Cytokines and Growth Factors—Cytokines and growth factors present in SF are important regulatory factors for the cell populations lining and within the synovial joint space, including chondrocytes and synovial cells.[6, 9] Regulatory molecules in SF may be derived from plasma or as secreted products of chondrocytes, synovial cells, and other cells within the SF or surrounding tissues. Cytokines may be categorized as pro-inflammatory or anti-inflammatory according to their predominant tissue-specific effects. Pro-inflammatory cytokines in SF include IL-1α, IL-1β, TNF-α, leukemia inhibitory factor (LIF), IL-6, IL-8, IL-17, and IL-18.[7, 47, 48] Anti-inflammatory cytokines in SF include IL-4, IL-10, and IL-13. Growth factors found in SF include TGF-β1 and insulin growth factor 1 (IGF-I) and have anabolic effects. Binding proteins are also present in SF and play important roles in cell regulation.

With joint injury and disease, the cytokine profile in SF is altered. Most cytokines and growth factors are at relatively low concentrations in normal SF, and are markedly elevated in joint injury and disease. Cytokines play an important role in disease pathogenesis and acceleration of joint destruction, and have attracted attention as potential therapeutic targets, with some cytokine-directed therapies already in clinical use and others in clinical trials.[8, 49] Some pre-clinical studies have demonstrated efficacy in improving synovial joint lubrication. Blocking the effects of TNF-α with etanercept, for example, resulted in increased amounts of lubricin bound to cartilage and decreased sGAG release from cartilage compared with untreated groups in a rat ACL injury model of post-traumatic arthritis.[50] Binding proteins such as IL-1 receptor antagonist (IL-1RA) can play chondroprotective roles in the synovial joint and are found at relatively high levels in normal SF, with time-dependent changes in their concentrations after ACL injury.[51, 52]

1.B.iv. Proteolytic Enzyme Activity—Degradative processes in the synovial joint are mediated primarily by proteolytic enzymes that are carefully regulated, as reviewed by Poole.[76] Matrix-degrading enzymes, such as matrix metalloproteinases (MMPs) MMP-1 and MMP-3, are present in normal SF and elevated in joint injury and disease.[65, 66] MMPs are

secreted primarily from chondrocytes as latent zymogens with propeptide domains that are cleaved during extracellular activation. A distintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) proteinases degrading aggrecan are also secreted as latent zymogens requiring subsequent activation. Other proteinases, such as serine and cysteine proteinases, are involved in activation of proMMPs. For example, plasmin, kallikrein, and cathepsin B activate proMMP-1. Tissue inhibitors of metalloproteinases (TIMPs) and inhibitors of proteinases that activate proMMPs are also present. In RA, polymorphonuclear leukocytes and synovial cells also contribute to proteinase levels in the joint. Changes in the levels and activities of matrix-degrading enzymes, and their associated inhibitors and activators, alter the balance between anabolism and catabolism in joint injury and disease. Changes in proteolytic activity in the synovial joint are indicated by elevated levels of degradation products. Concentrations of fragments of aggrecan and type II collagen are elevated in the SF of patients soon after joint injury and in patients with OA.[69, 77]

1.B.v. Cells—Normal SF is relatively acellular compared to whole blood, containing <200 leukocytes per mm^3 [78-80] compared to 3,540–9,060 per mm^3 in whole blood.[81] Of the nucleated cells in SF, only a minor percentage can be identified as synovial cells.[78, 79] Erythrocytes are virtually absent in normal SF, compared to $4.0–5.6 \times 10^6$ per mm^3 in whole blood.[81] OA and RA are usually accompanied by increased synovial effusions. The mean leukocyte count in RA SF is ~11,000–19,000/mm^3 [79, 82] compared to <2,000/mm^3 in non-inflammatory SF in OA and traumatic arthritis.[80] Lymphocytes, macrophages, and shed lining cells are also present in SF. Few neutrophils are found in RA synovium, even though they often comprise the majority of cells in the effusions.[83] Erythrocytes may be introduced into SF in cases of trauma.

1.C. SF Formation and Transport through Synovium

1.C.i. SF Formation—SF formation is a process that involves the functioning of synovium as a semi-permeable membrane that is size-selective. SF is an ultrafiltrate of blood plasma with additional molecules secreted by cells lining and within the synovial joint space. SF lubricant molecules are derived from the cell populations lining the synovial cavity, with HA secreted mainly by synoviocytes and PRG4 molecules by chondrocytes in the superficial zone of cartilage,[3]synoviocytes,[4, 36, 84] and meniscal cells.[85] Molecular sieving by the synovial membrane matrix is primarily size-dependent.[86] High molecular weight species within SF, which include the lubricant molecules HA and PRG4, are selectively retained within the synovial joint, while low molecular weight species, such as most metabolic substrates and byproducts, cytokines, and growth factors, are not. High molecular weight species within plasma, on the other hand, are unable to pass through the synovial membrane easily and are absent or low in normal SF. Joint injury and disease affecting the synovial membrane may impair the SF formation process, resulting in pathologic SF.

1.C.ii. Transport Characteristics of Synovium—Synovium is the main barrier to the transport of molecules in SF and plasma, such as lubricant molecules and plasma proteins, into and out of the synovial joint. Synovium is a vascularized, thin sheet of connective tissue with fibroblast-like (type B) cells and macrophage-like (type A) cells within an extracellular matrix (ECM) composed primarily of HA, collagen, and proteoglycans. The blood-joint barrier has been modeled as a double barrier, in series, consisting of synovial interstitial space that limits diffusion of small molecules, and microvascular endothelium that limits transport of proteins.[19, 87] Fluid flow through normal rabbit synovium occurs through interstitial spaces that range from 0.1–11.8 μm and average ~2 μm,[88] which has a hydraulic conductivity of $\sim 10^{-14}$ $(m^3 \cdot s)/(kg)$.[70] Diffusive and convective passive transport of molecules occurs by size-selective molecular sieving through synovial ECM, and can be

modeled as transport through pores with a calculated pore radius of ~25–60 nm.[86, 89] High MW lubricant molecules are selectively retained in the joint, as the reflection coefficient of synovium to high MW HA is ~57–75%.[90]

In joint injury and disease, alterations in synovium result in pathological SF. In RA, synovium increases dramatically in mass and metabolic activity, with redundant folds, frond-like villi, infiltration with immune cells, and edema. The synovial intimal lining, which directly contacts the synovial fluid compartment, increases greatly in depth up to 10-fold, although there are no tight junctions or basement membranes that interfere with the flux of fluid. Intimal hyperplasia is due to increased numbers of fibroblast-like synoviocytes and macrophage-like synoviocytes. The former express the adhesion protein cadherin-11, which accounts for aggregation of synoviocytes. The intimal lining is the location where most pro-inflammatory cytokines and metalloproteinases are produced in RA. The sublining is infiltrated with T cells, B cells, macrophages, and plasma cells, with CD4+ T lymphocytes as the most prevalent cell type. Lymphocytes can organize into aggregates and lymphoid follicles in ~20% of patients, with diffuse mononuclear cell infiltration or acellular fibrous infiltration occurring in others. Considerable variation exists throughout the synovium, necessitating careful experimental sampling. An increase in the number of blood vessels is usually prominent, although the capillary network tends to be more disorganized than normal.[83]

Molecular transport through synovium is markedly altered in diseased synovium. Synovium from joints of RA patients are more permeable to large proteins.[19, 87] Increased synovial inflammation in patients with various form of arthritis is associated with proportionately greater increases in permeability to larger plasma proteins.[91] Whereas permeability of RA synovium to large proteins is increased, permeability to small molecules (e.g., urea, glucose) is slightly decreased. This may be explained by a combination of increased vascular permeability and synovial hyperplasia and cellular infiltration. Increased Starling pressure due to the reduced oncotic gradient as protein accumulates in SF, increased permeability of capillaries, and an increase in capillary pressure during inflammation contribute to the buildup of fluid and protein in RA SF. HA contributes to outflow buffering in the synovial joint. In arthritis, the size of SF HA is reduced, with an increased rate of HA loss from SF.[86] Although synovial membrane inflammation is also recognized as a key factor in OA pathophysiology, the transport characteristics of synovium in OA are less affected than in RA, with apparent permeabilities to proteins over a range of sizes (molecular radii ~3–9 nm) over 3-fold higher in RA compared to OA.[92]

1.D. Rheology, Tribology, and Mechanobiology

1.D.i. Rheological and Tribological Properties of SF—The rheological and tribological properties of SF have been characterized through measurements of viscosity, viscoelasticity, and friction. Normal SF is a viscous, non-Newtonian, thixotropic[93] fluid. The rheological properties of SF are in large part attributable to the concentration and molecular mass of HA.[94, 95]Lubricin appears to play an important role in organizing HA and providing SF with the ability to dissipate strain energy.[96] SF behaves as a viscous material at low frequencies of oscillation, and as an elastic material at high frequencies.Studies with cartilage-on-cartilage friction tests have identified HA and PRG4 as important boundary lubricants in SF that lower friction in a dose-dependent manner, both alone and in combination.[97, 98] Cartilage-on-glass friction testing has revealed that recombinant human lubricin lubricates cartilage in a dose-dependent manner and by two distinct mechanisms, involving lubricin bound to the surface, and in solution.[99]

Pathological SF exhibits impaired rheological properties. Normal and OA SF display shear-thinning behavior, while RA SF displays Newtonian behavior.[95] Viscosity of normal SF is

higher than that of OA SF, which is higher than RA SF. In ACL-transected guinea pig knees, increased coefficients of friction and decreased lubricin concentrations were observed.[100] In ACL- and PCL-transected rabbit knees, lubricin concentrations and lubricating ability decreased, and collagen degradation increased, at weeks 2 and 3 after injury.[42] The lubricating ability of OA SF is not significantly different compared to normal SF, but RA SF lubricates poorly compared to normal SF.[101]

1.D.ii. Mechanobiology of Lubricant-Secreting Cells—Lubricant-secreting cells of synovial joints are regulated by certain mechanical cues. HA secretion by rabbit synoviocytes increases when primary cultures are subjected to stretch,[102] and when rabbit knee joints are exposed to passive cycling.[103] *HAS* and *PRG4* mRNA expression and HA and PRG4 release into conditioned media increase when scaffolds seeded with bovine chondrocytes are subjected to articulation.[104] PRG4 secretion by bovine chondrocytes also increases when cartilage explants are loaded with dynamic shear superimposed upon static compression[105] as well as when chondrocytes in three-dimensional culture are subjected to articulation loading;[104, 106, 107] in contrast, PRG4 secretion is inhibited by static compression alone.[105] The mechanism by which shear loading of cartilage stimulates PRG4 secretion appears to involve signaling pathways mediated by TGF-β.[108] Continuous passive motion applied to bovine joints increased PRG4 secretion and the percentage of chondrocytes expressing PRG4 in regions of the femoral condyle that experienced mechanical stimulation from continuous or intermittent sliding against apposing tissues.[109]

2. Systems Biology of the Synovial Joint

2.A. General Overview

As described in earlier sections, the synovial joint is composed of several cell and tissue types, which are altered in injury and disease. The study of isolated components and their changes can provide detailed information at the molecular, cellular, and tissue scales. However, insights into the behavior and properties of the synovial joint lubricant system require an overall consideration of components and their interactions. A systems biology approach can be employed to study how the relationships between the various components of a synovial joint contribute to produce a load-bearing and friction-lowering joint that, in most people and under normal conditions, provides a lifetime of normal joint function.

The pathophysiology of the most common rheumatic disorders, OA and RA, and joint injury, involves multiple changes at the molecular, cellular, tissue, and organ levels[110] (Figure 1). The normal, healthy, synovial joint involves cartilage, synovium, SF, and other tissues functioning together (Figure 1A). OA is a complex, heterogeneous disease associated with alterations in cartilage degradation and repair, synovial inflammation, and increased production and SF levels of catabolic and pro-inflammatory cytokines (Figure 1B).[111] The altered cytokine environment can enhance cartilage breakdown, which in turn may amplify synovial inflammation, leading to a cycle of events resulting in progressive joint destruction. OA develops progressively, but inflammatory flares can occur.[111] RA is a systemic inflammatory disease with features of synovitis, pannus formation, and SF volume increase, along with changes in the mass and concentration of plasmaproteins, cytokines, and key proteases such as MMPs and their proenzyme activators and inhibitors (Figure 1C). Acute, traumatic joint injury, such as to the anterior cruciate ligament, result in a number of mechanical and biological changes over time that can contribute to the development of post-traumatic OA (Figure 1D).[13-15] Post-traumatic OA is a subset of OA that is associated with an acute traumatic event. Some of the changes that may be involved in post-traumatic OA pathogenesis include increased cell death, increased levels of inflammatory cytokines and ECM-degrading enzymes, and decreased levels of lubricant molecules. Joint injury initiates events that, in many cases, lead to joint destruction, pain, and disability, with pathology and

clinical symptoms that are similar to primary OA. In injury and disease states, analysis of whole joint changes can provide an understanding of how joint pathophysiology arises from the interplay between component parts and contributes to the disease phenotype. Following the systems biology paradigm, data on multiple scales and from various sources can be integrated into models that predict emergent properties of the biological system. For the synovial joint lubrication system, this approach may involve integration of transcriptome and proteome data with physicochemical tissue descriptions to construct models that account for the complex interactions between cartilage, synovium, and SF in the synovial joint. At the tissue and whole joint level, physicochemical principles of mass balance and transport, combined with data from transport studies, can be integrated into the mathematical framework of a model. Once constructed, models can be a powerful simulation tool to predict the behavior of the biological system under different conditions, generate new testable hypotheses, and provide insights into the physiology and pathophysiology of the synovial joint.

2.B. Data Types and Sources

2.B.i. Conventional Methods—Conventional methods of collecting experimental data provide detailed information about specific entities. ELISA assays, for example, provide valuable quantitative data on specific molecules, such as cytokines and lubricant molecules, in the synovial joint under different conditions. Concentrations of molecules of interest in SF, measured by conventional methods, may be a valuable resource for estimating values of parameters in models of the synovial joint (Table 1A,B). Such methods, however, are typically applied to study a few entities of interest, although multiplex variants of such methods allow study of multiple targets simultaneously. As study of the synovial joint shifts from specific molecules and isolated components to a more comprehensive, systems biology approach, methods that enable the study of many components will be useful.

2.B.ii. Large-Scale, High-Throughput Methods—The application of large-scale, high-throughput technologies to synovial joint tissues is leading to an abundance of data on components of the synovial joint system. Transcriptomic studies aim to characterize gene expression on a large scale, on the order of thousands of genes, in order to capture the global state of the cell. Commonly used microarray technologies include Affymetrix chips and Illumina platforms and enable high-throughput screening by determining the expression of tens of thousands of genes in a massively parallel manner. Much of this data is accessible as datasets in peer reviewed, public repositories such as the Gene Expression Omnibus from the National Center for Biotechnology Information and ArrayExpress from the European Bioinformatics Institute. Several datasets of samples of synovial joint tissues and cells have been published.[112-120]

Common methods for the large-scale characterization of proteins in synovial tissues include two dimensional polyacrylamide gel electrophoresis (2D-PAGE) or liquid chromatography for protein separation and mass spectrometry (MS) for protein identification. Several software packages are available for spot detection, matching, and quantitation of 2D-PAGE images, and comparisons between two widely used ones, PDQuest and Progenesis, have been made for the analysis of SF samples.[121] Advances in protein microarray technologies add another tool for proteomic studies of synovial tissues.

A number of biological issues can complicate the interpretation of data from high-throughput methods, and guidelines for evaluating microarray studies have been suggested.[122] Transcriptomic and proteomic studies on complex tissues, such as RA synovium, can be complicated by variation between samples and other issues such as heterogeneity of the cell population in native and diseased tissues. One approach to

circumventing this challenge is to focus on individual cell types isolated from tissues. In the case of RA synovium, for example, RA synovial fibroblasts can be studied after being isolated from synovium.

Extraction of biological information from the large volumes of data provided by large-scale and high-throughput methods has been achieved in several ways. Significance Analysis of Microarrays is a statistical technique for determining whether differentially expressed genes in microarray experiments are statistically significant.[123] The Significance Analysis of Microarrays method has been applied to identify sets of genes that are differentially expressed in subgroups of RA synovium[124, 125] and fibroblast-like synoviocytes,[126] providing evidence of the heterogeneous nature of RA. Clustering methods, such as hierarchical and k-means clustering, group together genes that are co-expressed under different conditions, to identify functionally related genes. Principal component analysis reduces the dimensionality of a dataset. Clustering analysis and principal component analysis have been used to identify patterns of gene co-expression by cartilage explants and possible pathways involved under different mechanical loading conditions.[127-129] For example, transcription of most matrix proteins is upregulated by dynamic compression and dynamic shear, but downregulated by static compression, while most matrix proteases are upregulated under all three loading conditions.[128] Data from high-throughput gene expression studies may be analyzed by gene set enrichment analysis for pathway level analysis, by associating gene expression data with various pathways. Gene set enrichment analysis determines whether sets of genes of pre-defined pathways or processes are enriched or impoverished in a candidate gene list.[130]

The experimental data from large-scale analyses are useful screens to characterize disease by identifying key molecular constituents that may be mediators of disease on the basis of correlated molecular behavior observed in the datasets. For example, high-throughput methods have suggested the potential pathogenic role of a number of genes and proteins not previously associated with OA, on the basis of differential expression of those genes and proteins (Table 2A,B). Data from high-throughput methods, in particular those concerning key molecules, may be validated with conventional, low-throughput methods such as real-time RT-PCR for quantifying mRNA levels, and ELISA and Western blot for measuring protein. Profiling gene and protein expression patterns of synovial joint tissues has identified key molecular constituents associated with joint injury and disease and provided insights into the molecular basis of synovial joint changes in health, injury, and disease. Important features of the system as suggested by these data sets may then be integrated into model descriptions of joint homeostasis.

2.C. Transcriptomic Studies

2.C.i. Synovium and Synovial Cells—In genome-wide gene expression profiling of synovial cells from OA and RA patients, many differentially regulated pathways have been identified, with some validated by conventional techniques such as real-time RT-PCR and Western blots. In RA synovial fibroblasts, upregulation of TGF-β pathway components[114] and pathways involved in protection against oxidative stress,[136] in particular thioredoxinreductase 1, have been reported.

Gene expression profiling can further be used to examine the effects specific cytokines can have on the synoviocyte transcriptome. Stimulation of synoviocytes with TNF-α, which is important in the pathogenesis of RA, induced elevated *Naf1* gene expression in RA compared to OA, suggesting a possible role of *Naf1*, an inhibitor of NFκB-dependent gene expression, in modulating TNF-α activity in RA.[137] In addition, clusters of RA related genes, involved in inflammation, synovial proliferation, and bone and tissue destruction, were upregulated by PGI2-IP signaling in mouse synovial fibroblasts subjected to collagen

induced arthritis, suggesting a role for PGI_2-IP signaling in RA.[138] Gene expression studies on synoviocytes under the influence of TNF-α alone, IL-1β alone, TNF-α and IL-17 together, and TNF-α and IL-1β together, have found that these cytokines induce the same genes that are upregulated in patients who respond poorly to adalimumab therapy, suggesting a possible role of these cytokines in drug resistance.[112]

Gene expression is different in synovium from normal donors and OA and RA patients. Analysis of inter-individual, intra-group gene expression variances within normal donors and OA and RA patients has identified several potentially disease relevant pathways and complexes by comparing genes with different variances in different patient groups.[120] Many of the identified pathways for RA are involved in inflammation, angiogenesis, proliferation, and cell survival. Synovial membranes from OA patients showed higher gene expression variances in genes involved in pathways such as oxidative phosphorylation, MAPK signaling pathway, folate biosynthesis, and starch and sucrose metabolism, representing a desynchronization of metabolic processes. Synovial membranes from RA patients, on the other hand, were found to have increased gene expression variances in pathways involved in melanogenesis, B-cell receptor signaling, and VEGF signaling. Microarrays have shown differential gene expression profiles in synovium from RA patients, identifying two molecular subtypes of RA synovium, with one subgroup upregulating clusters of genes involved in immune activation pathways and the other upregulating genes, such as collagens and *SOX9*, suggesting fibroblast dedifferentiation.[124] Synoviocytes with molecularly distinct expression patterns in synovium have been reported in RA, with an association between α-SMA expressing myofibroblast-like synoviocytes and highly inflamed synovial tissue.[125, 126] Distinct expression profiles have also been observed in early compared to late RA, suggesting different biological processes and pathways may be involved with RA disease progression.[119]

2.C.ii. Cartilage and Chondrocytes—Gene expression profiling has been used to study chondrocyte transcriptome in health, injury, and disease. Chondrocytes from OA patients compared to those from patients undergoing autologous chondrocyte transplantation for focal cartilage lesions are less differentiated in monolayer culture, as assessed by gene expression profiling, but the number of differentially expressed genes decreases following chondrogenic differentiation in 3D culture scaffolds, with *TIMP3* the most notable persistently differentially expressed gene.[113]

Determining expression profiles in response to specific manipulations *in vitro* is one approach to elucidate important molecular mediators in signaling pathways relevant to joint disease. In OA and RA, TNF-α levels in SF are elevated. Microarray technology has been used to study the effects of TNF-α stimulated MEK/ERK signaling on global chondrocyte gene expression, resulting in the discovery that among the TNF-α modulated genes, proinflammatory genes are MEK/ERK independent, while genes encoding proteins with proteinase activity and HA binding activity, contributing to matrix catabolism, are MEK/ERK dependent.[115] Further studies showed that TNF-α regulation of the transcription factors SOX9 and NFκB are MEK/ERK independent, whereas regulation of EGR-1 is MEK/ERK dependent. Another cytokine implicated in OA and RA, IL-1β, has been studied for its effects on chondrocyte gene expression.[116] Chondrocytes stimulated by IL-1β increased expression of GCSF, SELE, LIF, IL-1β, IL-6, IL-8, and a number of chemokines, and decreased expression of type II collagen and ADAMTS-5. OA cartilage had gene expression patterns similar to normal cartilage stimulated with low dose IL-1β. IL-1β induced altered gene expression in chondrocytes were partially reversed by TGF-β1, but not by BMP-2.[116]

A number of transcriptomic studies have been conducted on cartilage tissue. In experimental models of OA in rats, 1619 significantly differentially expressed probe sets were found compared to control, including genes expressed in hypertrophic growth plate chondrocytes genes for cytokine and chemokine signaling.[132] Newly identified genes with potential roles in OA pathogenesis were identified, including chemokine signaling factors *CXCR4*, *CKLF1*, *CX3CL1*, and *CTSC*. Comparing cartilage from OA and normal donors, new genes not previously associated with OA, including some involved in bone formation and collagen synthesis,[131] were found upregulated in OA samples (Table 2A). *TOB1*,[133] which is an anti-proliferative factor not previously associated with OA, *SOX6*, and *SOX9*[139] were repressed in OA. The role of different degradation pathways in late vs. early OA have been suggested by microarray analysis showing downregulation of *MMP3* and upregulation of *MMP2* and *MMP13* in late-stage disease.[140] Comparisons of intact and damaged regions of OA cartilage have revealed upregulation of genes involved in wound healing, cell proliferation, and collagen synthesis in damaged regions.[141] Cartilage samples with induced mechanical injury compared to normals showed differentially expressed genes, including upregulation of *WNT16* upon injury.[142] Approximately 30% of the differentially regulated genes in OA vs. normal were also altered in injury vs. normal.

2.D. Proteomic Studies

2.D.i. Synovial Fluid—The characterization of protein and peptide components in SF in a comprehensive and high-throughput manner has provided investigators with another tool to study the underlying pathophysiology of joint injury and disease. Endogenous peptides are of interest because they are major regulators of biological functions in health and disease. Cytokines and growth factors, for example, are critical in cell signaling and metabolism, and alterations in their abundance and activity levels in the synovial joint and their role in injury and disease have been described earlier. Additionally, peptides and peptide fragments may serve as surrogate markers of enzymatic and proteolytic activity, with potential applications as biomarkers to diagnose, prognosticate, or monitor disease progression and treatment. One approach to discovering sets of proteins associated with joint injury or disease is comparative proteomics, which aims to detect and characterize the differences in abundance and activities of expressed proteins and peptides in samples of SF from donors in normal and injured or diseased states. These systems-wide analyses have confirmed the involvement of previously known participants and opened new lines of investigation by implicating novel and previously unassociated molecular pathways and mediators that contribute to joint disease.

In recent years, several proteomic studies of SF have contributed insights into the molecular mediators of joint disease. Several peptide cleavage products from proteins previously associated with OA were elevated in SF from OA patients compared to SF from cadavers without joint disease, including type II collagen, PRG4, serum amyloid A (SAA), tubulin, vimentin, and matrix gla protein.[143] In studies of SF and plasma from patients with RA, OA, or reactive arthritis, calgranulin B was exclusively identified in RA SF, and SAA was identified in RA SF, but not OA SF.[144] Comparing the SF proteome in health and OA, cystatin A, aggrecan, and dermicidin levels were lower in OA SF compared to healthy SF.[145] No duration-dependent changes or disease-stage specific differences were found in OA SF, but two distinct patterns of protein expression were observed. Proteomic studies have identified biomarkers of disease severity, with C-reactive protein, S100A8, S100A9, and S100A12 elevated in the SF and serum of patients with erosive RA compared to nonerosive RA or normal.[146, 147]

2.D.ii. Cartilage and Chondrocytes—The proteome of normal human chondrocytes has been characterized by 2D-PAGE and MS under basal and stimulated conditions. A reference

map showing 136 spots and 93 identified proteins is available.[148] The proteome of normal chondrocytes in response to cytokine stimulation has been investigated.[149] Stimulation by IL-1β differentially modulated 22 proteins involved in cytoskeletal rearrangement and cellular metabolism. TNF-α modulated 20 proteins involved in transcription, synthesis, and protein turnover, cytoskeletal rearrangement, and stress responses. The combination of both cytokines modulated 18 proteins related to metabolism and energy and transcription, protein synthesis, and turnover. Nine novel proteins not previously known to be synthesized by human chondrocytes were discovered in cytokine-stimulated chondrocytes.

Proteomic analyses have also been applied to the study of conditioned media from cultured explants of normal and injured or diseased cartilage. In one study, conditioned media was analyzed in order to characterize the proteins that are secreted and released by osteoarthritic cartilage.[150] Proteins previously known to be secretion products of cartilage, such as YKL-39 and osteoprotegerin, were identified, along with a number of novel proteins not previously known to be secreted by cartilage, such as serum amyloid P-component, pigment epithelium derived factor, fibrinogen, lyl-1, thrombopoietin, fibrinogen, angiogenin, gelsolin, and osteoglycin. Medium from explants subjected to injurious compression, IL-1β, or TNF-α contained ECM proteins such as aggrecan, collagens, perlecan, fibronectin, and link protein. Novel cartilage proteins were also identified, including CD109, platelet-derived growth factor receptor-like protein, angiopoietin-like 7, and peptidoglycan-recognition protein long. CHI3L1, CHI3L2, complement factor B, MMP-3, ECM-1, haptoglobin, serum amyloid A3, and clusterin were increased in the medium of explants treated with IL-1β and TNF-α. Type VI collagen subunits, cartilage oligomeric matrix protein, and fibronectin were increased in explants subjected to injurious compression. Proteins involved in inflammation and stress response were increased in the medium of IL-1β and TNF-α treated explants. Release of intracellular proteins into the medium was increased in compression-injured explants, suggesting cell death and loss of cell membrane integrity.[151]

Proteomic approaches may be applied to the study of the proteomes of different cellular components of chondrocytes. The proteome of articular cartilage vesicles was characterized in one study.[152] 170 proteins were identified in articular cartilage vesicles from cartilage of 10 OA and 10 normal patients. A number of proteins were discovered with significantly different levels in OA and normal patients. Six proteins were found exclusively in vesicles from normal cartilage, and 9 proteins, including inflammation related proteins such as fibrinogen, complement, immunoglobulins, and apolipoproteins, were found exclusively in vesicles from OA cartilage. In another study, the mitochondrial proteome from chondrocytes isolated from healthy donors was obtained by 2D-PAGE and MS and a reference map was constructed.[153]

Recently, a method for the selective extraction of proteins from cartilage has been described, allowing direct proteome characterization of human articular cartilage by 2D-PAGE and MS.[154] To study differences in protein synthesis in cartilage from OA patients and normal donors, cartilage explants were incubated in medium containing [^{35}S] methionine/cysteine to label newly synthesized proteins and analyzed.[134] Collagen II and activin A were strongly upregulated in OA cartilage compared to controls. Analysis of normal cartilage from 7 donors and OA cartilage from 7 OA patients revealed 59 proteins that were differentially expressed, including HtrA serine protease 11 which was found at levels 8 fold higher in OA compared to normal cartilage. A number of proteins not previously associated with OA were also identified (Table 2B), including fibulin 3, osteonectin, secreted modular calcium-binding protein 2, vitrin, and tenomodulin.[135]

2.D.iii. Synovium and Synovial Cells—Proteomic approaches have been used to characterize protein expression in synovial cells isolated from healthy donors and patients

suffering from OA and RA. Analysis by 2D-PAGE and MS of synovial cells from RA patients confirmed the presence of a number of proteins previously known to be important in synovial cell function. From this study, a protein product of chromosome 19 ORF10 was identified, whose biological activity and role in synovial cell function is unclear.[155] In synovial fibroblasts obtained from 6 OA, 5 RA, and 7 control patients, 2D-PAGE revealed 15 and 34 proteins that were significantly elevated in OA and RA synovial fibroblasts, respectively, compared to controls.[156] Validation by Western blot showed that levels of enolase-α, S100A4, S100A10, annexin I, cathepsin D, mitochondrial superoxide dismutase, and peroxiredoxin 2 were significantly higher in OA and RA synovial fibroblasts compared to controls, while mitochondrial manganese superoxide dismutase and cathepsin D were significantly higher in OA compared to RA synovial fibroblasts.

A few studies have investigated the proteome of synovium from OA and RA patients. 2D-PAGE of synovium from 6 OA and 6 RA patients showed that 5 gel spots were significantly differentially expressed.[157] Hierarchical cluster analysis showed RA patients to cluster separately from OA patients, and OA patients to be divided into two subclusters. Levels of calgranulin A were significantly higher in synovial tissues from RA patients compared to OA patients, and validated by real-time RT-PCR in a separate cohort of 10 OA and 9 RA patients. Using a panel of 791 mouse antibodies, the proteins expressed in synovium samples from one OA and one RA patient were compared, with 260 antibodies detecting their target proteins in the samples.[158] Levels of 71 proteins differed significantly between the OA and RA patients. Validation with Western blots of 8 OA and 8 RA patients showed that protein levels of Stat1, p47phox, and MnSOD were significantly increased, and cathepsin D decreased, in RA synovial tissue compared to OA.

2.E. Interactions between Joint Tissues

The production and function of SF involves interactions between different joint tissues, cytokines, and the physical transport processes that connect all joint components. As new mechanistic details of SF, joint tissues, and cytokine effects are discovered, a comprehensive model of synovial fluid in health, injury, and disease will be needed to account for how the interactions between components give rise to joint function and behavior.

Interactions and regulatory influences between various synovial joint tissues play an important role in joint health and disease. The involvement of synoviocytes in the breakdown of cartilage in inflammatory arthritis has long been recognized,[159] with the finding that synovial tissue culture medium contains a catabolic protein capable of inducing chondrocytes to degrade cartilage ECM.[160] More recently, the effects of RA synoviocyte metabolic products on chondrocyte gene expression have been studied *in vitro* by culturing chondrocytes in alginate beads suspended in conditioned medium from RA synovial fibroblasts, normal synovial fibroblasts, and anti-rheumatic drug treated synovial fibroblasts.[117, 118] Chondrocytes cultured in RA synovial fibroblast conditioned medium, compared to normal synovial fibroblast conditioned medium, differentially expressed 110 genes, upregulating genes associated with immunological and catabolic processes and downregulating genes associated with cell proliferation and differentiation.

Responses to cytokines are specific to tissues, cell types, and culture conditions, and may be influenced by interactions between multiple cytokines. TGF-β1 generally upregulates HA secretion by normal synoviocytes.[6, 161] In chondrocytes and synoviocytes, TGF-β1 and IL-1β increased HA secretion, individually and synergistically.[162] In rheumatoid fibroblastic synovial lining cells, IL-1β stimulates and TGF-β1 inhibits HA synthesis.[163] Three isoforms of hyaluronan synthase (HAS), the enzyme responsible for HA synthesis, have been identified, and HAS1 mRNA predominates in synovial cells, whereas HAS2 mRNA predominates in chondrocytes.[162] TGF-β1 has a differential effect on HAS in human

fibroblast-like synoviocytes, upregulating HAS1 and downregulating HAS3 in a dose dependent manner.[164] Cytokines in SF modulate PRG4 as well, as TGF-β1 upregulates and IL-1βdownregulates PRG4 gene expression and protein secretion in superficial zone cartilage, synovium, meniscus, and the anterior and posterior cruciate ligaments.[165] The effects of cytokines on cartilage matrix and chondrocyte metabolism have also been studied, with IL-1α, IL-1β, and TNF-α inhibiting proteoglycan synthesis and increasing proteoglycan degradation, while IGF-1 and TGF-β1 had the opposite effects.[166]

2.F. From Data to Models

The systems biology approach culminates in the construction of a model, a simplified representation of the basis for a biological phenomenon. Data of multiple types from multiple sources are organized within a mathematical framework. The most useful models reproduce experimentally obtained data, provide quantitative predictions of the behavior of the system under new conditions, and serve as a platform for the generation of new hypotheses that may be experimentally validated, all of which may lead to further refinement of the model. Synovial joint phenomena that may benefit from a systems biology approach of study include processes such as the formation of synovial fluid and signaling networks, which involve the interaction of multiple component parts. Ultimately, models aim to provide insight into mechanisms underlying specific biological phenomena. Several models of the component parts of the synovial joint in isolation have been constructed, including models with a focus on the rheological properties of SF,[167-171] lubrication mechanisms,[172-174] and mass transport in cartilage[175-182] and synovium.[183-186] More recently, models of the whole joint with interacting components have been constructed to enable understanding of synovial joint function and behavior at the whole joint level.

2.F.i. Model Construction—Models aim to recapitulate some aspect of the properties of a biological system or process that is of interest to the modeler. In constructing models, the advantages of simplicity and complexity must be weighed with the primary modeling goal in mind. Simple, idealized models may provide clearer insight into the system and reveal the main features and relationships of the system. Complex, detailed models may provide increased predictive power of the behavior of the system, but often at the cost of understanding how the system functions.

Data and insights gained from the detailed study of isolated components are useful in the construction of mechanistic models of a process of interest. For example, studies from synovial tissues in isolation have provided information on the transport properties of synovium, which can be used to construct models of fluid and mass transport processes in synovium. Data from high-throughput screens can be used to refine and validate the model. Knowledge of key molecular constituents, processes, and pathways in the synovial joint that are altered in injury and disease may suggest relevant details and parameters to incorporate into models.

2.F.ii. Models of the Synovial Joint System—A few integrative models of the synovial joint have been developed recently and illustrate a systems biology approach to studying the synovial joint. As data from large-scale, high-throughput analyses and conventional analyses become integrated with current models, new models of the synovial joint are anticipated.

A quantitative, compartmental model of the synovial joint to predict steady-state SF lubricant concentrations in the human knee joint has been constructed.[187] The model consists of four compartments: (i) SF surrounded by (ii) articular cartilage and (iii) semi-permeable synovium, which provides the outflow resistance and filtration of SF to the (iv)

subsynovium. Lubricants are secreted into the SF compartment by chondrocytes in the articular cartilage compartment and synoviocytes in the synovium compartment. The governing equations of the model were formed by mass balances of the lubricant molecules HA, PRG4, and SAPL, and depend on rates of secretion, degradation, and flux across compartments. Values for the model parameters are found from a thorough search of the available literature. The system of linear, first order differential equations with three state variables is solved numerically with Matlab. Predictions of steady state concentrations of lubricant molecules under basal, TGF-β, and IL-1 conditions, and transient concentrations with simulated joint lavage and therapeutic HA injection were found to be consistent with experimental data.

A mathematical model of RA has been constructed with the EntelosPhysioLab® platform, with representations for inflammatory cells, endothelium, synovial fibroblasts, and chondrocytes within a synovial joint compartment.[188] Cytokines and growth factors, including TNF-α, IL-1β, IL-6, IFN-γ,TGF-β, GM-CSF, VEGF, chemokines, MMP proteases and TIMP inhibitors, and cell surface molecules are represented in the model. Program simulations can predict synovial hyperplasia, cartilage degradation rate, bone erosion index, and soluble factor concentrations. The model can be used to determine the critical pathways that produce the predicted disease outcomes, suggest potential new targets for pharmaceutical research, and evaluate the expected clinical efficacy of candidate pharmacological targets.

Conclusion

The synovial joint is a complex biological system composed of several highly specialized cell and tissue types. Normal joint loading and articulation depends on the precise interplay between the various components of a synovial joint. Pathologic alterations in joint injury and disease involve changes in multiple components across a range of scales. Developments in large-scale, high-throughput technologies provide an extensive enumeration of the changes that occur in joint injury and disease. Diverse data types from multiple sources can be integrated in defining the components and parameters of a mathematical model of the synovial joint system. A systems biology approach will be an invaluable tool to the study of joint behavior and function in health, injury, and disease.

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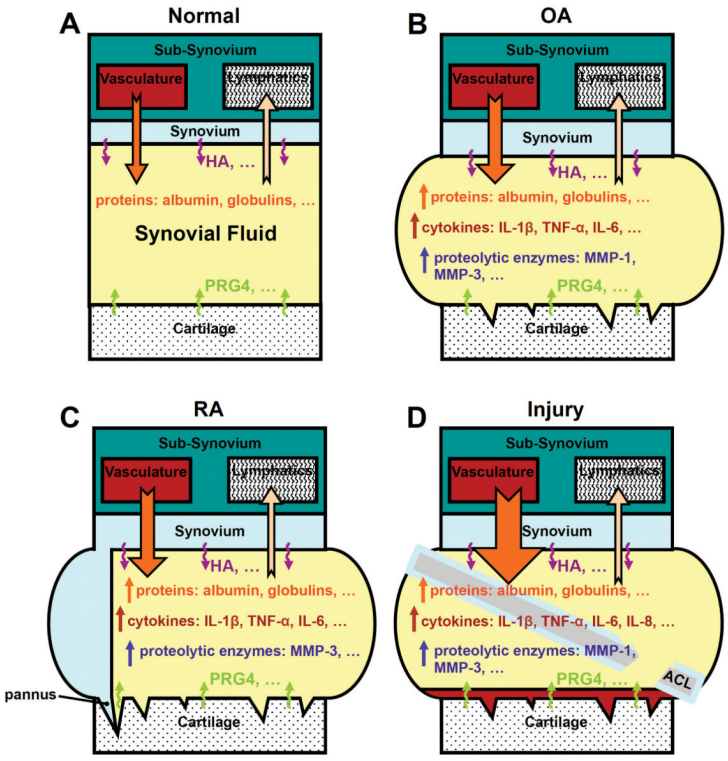

Figure 1.

Joint injury and disease involve pathologic changes in multiple joint tissues. Schematic view of synovial joint tissue changes in (B) OA, (C) RA, and (D) injury, compared to (A) a normal, healthy joint. Notched, outlined arrows represent fluid flows from vasculature to SF, and from SF to lymphatics. Undulating arrows represent secretion of lubricant molecules HA and PRG4 into SF. The relative sizes of arrows indicate relative magnitudes of flows and secretions. For example, increased flow from the vasculature to SF in (D) injury compared to (A) normal is represented by an orange, notched, outlined arrow that is larger in (D) injury compared to (A) normal. Up-arrows indicate increases in concentrations of the various substances.

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Table 1

List of concentrations of molecules of interest in healthy, arthritic, and injury synovial fluid. Data are shown as mean±SD, or *median*. *Concentrations in SF lavages.

		Synovial Fluid Concentrations in Various States			
		Healthy	**OA**	**RA**	**Injury**
Lubricant Molecules	HA	3.2±0.4,[27] 3.6,[28] 4.1±1.0[29] mg/ml	1.2±4.7,[29] 1.9±0.5,[27] 2.2[37] mg/ml	0.7±0.3,[29] 0.8±0.3,[39] 1.2±0.3,[40]1.2±0.4,[27] 2.7[37] mg/ml	1.1±0.4,[29] 1.7±1.0[27] mg/ml
	PRG4 SZP Lubricin	0.035±0.028,[32]0.16,[24] ~0.24[25]mg/ml	0.15±0.02[32] mg/ml	-	variable[31,43]
Signaling Molecules	IL-1α	~0*,[52] 6.6±1.0 [51] pg/ml	-	-	variable*[52]
	IL-1β	0±0,[169] ~5*,[52]10.0±4.3,[51]<20 [170] pg/ml	1.0,[171] 1.4±1.3,[172]21±13,[170]27.8±4.5[173]pg/ml	9.9±11.3,[172] 14.4,[174] 24.0,[171] 116±240,[175] 130±22,[173]193±57[170] pg/ml	variable[31,43,52,169,176,177]
	TGF-β	-	40.4±8.8[178](males),31.8±36.6[178](females) pg/ml	-	variable[176]
	TNF-α	0±0,[169]<2*,[52]<4.4,[51] 2900±900[170] pg/ml	4.8±3.0,[172] 50±40,[173] 80±30[170] pg/ml	19.5±15.1,[172] 101,[174] 170±40,[170]390±40[173] pg/ml	variable[31,51,169,176,177]
	IGF-I	-	60–80 ng/ml[178]	-	-
	IFN-γ	9±7.5 pg/ml,[169] 38.8±13 U/ml[170]	23.8±14.0 U/ml[170]	0.6±0.2 U/ml[170]	variable[169]
	IL-2	0±0[169]	5.2±0.6 U/ml,[173] 12.5±8.0 pg/ml[172]	4.5±0.6 U/ml,[173] 85.2±94.4 pg/ml[172]	variable[169]
	IL-4	0±0[169]	1.4±1.9 pg/ml[172]	1.4±1.7 pg/ml[172]	variable[169]
	IL-6	0±0,[169] 0.8±0.4 pg/ml[51]	3550±2950,[172] 212±85[178](males), 95±56 pg/ml[178](females), 490±324 U/ml[179]	0.99±1.01,[175] 9.6,[174] 16.0±9.9[172] ng/ml, 5290±1730 U/ml[179]	variable[31,51,169,176,177]
	IL-8	0±0,[169] 21.0±5.1[51] pg/ml	1.1±1.8 ng/ml[172]	0.236±0.412,[175] 21.9±36.8[172] ng/ml	variable[51,169,176,177]
	IL-18	36.2±17.4 pg/ml[180]	88.2±34.6 pg/ml[180]	278±65 pg/ml[180]	-
	GM-CSF	0.9±0.3,[51] 4±5[169] pg/ml	-	-	variable[51,169]
	PGE2	0.6±0.2 ng/ml[180]	2.8±1.4 ng/ml[180]	5.1±1.8 ng/ml[180]	-
	NO	22.6±14.8 μM[180]	57.5±29.4 μM[180]	131±36μM[180]	-
	PTHrP	-	1.8±0.2 pM[172]	2.6±0.9 pM[172]	-
Degradative Enzymes	ADAMTS-4	-	-	-	-
	MMP-1	*0.12*(pro, active, and bound) ng/ml[55]	*0.84*(pro, active, and bound),[55]*356*(free and pro)[54] ng/ml	-	variable[55]

		Synovial Fluid Concentrations in Various States			
		Healthy	**OA**	**RA**	**Injury**
	MMP-3	*6.7* ng/ml(pro, active, and bound),[55] *2* (pro, active, and bound),[181] 2.7(pro, active, and bound)[182] nM	*31.2* (pro, active, and bound),[55] *11,700* (pro, active, free, and bound)[54] ng/ml	88.6±78.5 ng/ml[175]	variable[43,55,56,181,182]
	TIMP-1 (free)	*6.8* ng/ml(free),[55] *4.8* (free),[181] 5.2(free)[182] nM	*19.7* (free),[55] *407* (free and bound)[54] ng/ml	1040±1410 ng/ml[175]	variable[55,56,181,182]
	TIMP-2	-	*165* (free and bound) ng/ml[54]	-	-
Binding Proteins	Albumin	~10 mg/ml[69]	-	~19 mg/ml[21,183]	-
	TNF-R	-	8.7±3.7 ng/ml[184]	21.0±9.3 ng/ml[184]	-
	IL-1RA	~67*,[52] 2520±1470 pg/ml[51]	614,[171] 486±258[178] (males), 333±106[178] (females) pg/ml	17.0 ng/ml[171]	variable[51,52,176,177]
	IL-2R	-	*606* U/ml[184]	*1670* U/ml[184]	-
	IL-18BP	179±86 pg/ml[180]	138.4±52.7 pg/ml[180]	56.5±25.8 pg/ml[180]	-
Structural Proteins	CPII	-	-	-	variable[158]
	CTXI	-	-	-	variable[158]
	CTXII	1.4[56] ng/ml	4.8[56] ng/ml	-	variable[43,56]
	sGAG	~0.1,[185] ~0.06[186] mg/ml	~0.08,[185] ~0.06[186] mg/ml	~0.04 mg/ml[185]	variable[31,43,51,186]
	ARGS aggrecan	*0.5*[186] nM	*4.6*[186] nM	-	variable[43,186]
	COMP	47[187] μg/ml	~90[187] μg/ml	-	variable[43,187]

Table 2

Genes not previously associated with OA implicated by high-throughput transcriptomic methods to be involved in OA pathogenesis.

Functional Classifications	Gene Symbols
Chemokines	*CCL3*[115]
	CCL4[115]
	CKLF1[114]
	CX3CL1[114]
	CXCL2[115]
	CXCL3[115]
	CXCL14[115]
	CXCR4[114]
Cytokines	*TNFRSF12A*[115]
	TNFRSF21[115]
Growth Factors	*FGF1*[115]
	LTBP1[115]
Matrix Components	*COL8A2*[115]
	COL13A1[115]
	COL14A1[115]
	COL15A1[115]
	ECM1[115]
	ECM2[115]
	EFEMP1[115]
	FBLN5[115]
	LAM4[115]
	SPON2[115]
Bone related	*CDH11*[115]
	CHST11[115]
	CLEC3A[115]
	CLEC3B[115]
	GPNMB[115]
	MSX1[115]
	MSX2[115]
Anti-proliferative factor	*TOB1*[116]
Degradative Enzymes	*CTSC*[114]
	MMP19[115]

Table 3

Proteins not previously associated with OA implicated by high-throughput proteomic methods to be involved in OA pathogenesis.

Proteins
Activin[133]
CTGF[133]
Cytokine-like protein C17[133]
Fibulin 3[134]
Osteonectin[134]
Secreted modular calcium-binding protein 2[134]
Tenomodulin[134]
Vitrin[134]